



Commentary

Proteasome inhibition by bortezomib: A left hook and a right punch



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Bortezomib (a.k.a. as velcade or PS-341) is used in the clinic to treat multiple myeloma (MM) and mantle cell lymphoma (Dou and Zonder, 2014). In addition to the pharmacokinetic properties of the drug, the effectiveness of the bortezomib depends largely on the difference in drug sensitivity of the target (cancer) cells compared with other cells in the body. For bortezomib this is particularly relevant as it targets a protein complex that is essential for life in all cells. Thus, it is of fundamental importance to understand why different cells show different levels of sensitivity to this drug. The work presented in this issue of *EBioMedicine* by Pitcher et al. (2015–in this issue), provides a new and intriguing explanation for the exquisite sensitivity of multiple myeloma cells to bortezomib.

Bortezomib targets one of three active sites of the proteasome. This was already known before it was developed as drug and has been confirmed through crystallization studies, showing in molecular detail that bortezomib interacts with the chymotrypsin-like active site in the proteasome, subunit PSMB5 (Borissenko and Groll, 2007). Proteasomes are important for the degradation of short-lived proteins in the cell. As a consequence proteasome activity is crucial for many cellular processes, ranging from cell cycle regulation to signal transduction pathways and from DNA repair to antigen presentation by MHC class I. Despite a wealth of knowledge concerning these different aspects, we still don't understand why low levels of proteasome inhibitor are lethal for MM cells, whereas other cells are much more tolerant of proteasome inhibitor. One early rationale was that MM cells depend more than other cells on the NF- κ B pathway, where I κ B α needs to be degraded by the proteasome. However, this does not explain the difference in sensitivity (Hideshima et al., 2009). An alternative explanation is that the proteasome in MM cells has a much higher workload as compared with other cells (Bianchi et al., 2009). As a result modest inhibition would directly be detrimental for this cell type, while other cell types can buffer this reduced capacity. Neither these, nor other explanations put forward so far, have been fully satisfying in light of published literature (Dou and Zonder, 2014; Kubiczкова et al., 2014). Identifying the factors that contribute to the differences in sensitivity is important, as it would greatly facilitate drug development as well as help understand and predict patient responses to bortezomib treatment for MM and other cancers.

The work by Pitcher et al. starts with the observation that challenging an MM cell line with a lethal 10 nM concentration bortezomib causes an almost complete inhibition of proteasome activity, as measured with the peptide substrate LLVY-amc. Control cell lines, to which 10 nM bortezomib is not lethal, showed a substantial higher level of remaining proteasome activity. Thus, the mechanism responsible for severe inhibition might provide important clues towards the specificity and efficacy of proteasome inhibitor drugs.

The observed severe inhibition in MM cells is surprising, because lysing the cells prior to treatment with the same concentration of bortezomib only resulted in a 40% reduction in activity. After eliminating potential trivial explanations, the authors reach the conclusion that specifically in living cells proteasome inhibitor treatment results in some indirect enhanced inhibition. The authors identify changes in a post-translational modification for several proteasome subunits. This modification, which remains to be fully characterized, has previously been reported by the same group (Pitcher et al., 2014). It appears to be mainly nuclear, have unusual biochemical properties, and similarities to ADP ribosylation. As the modification remains somewhat elusive, manipulating the levels of modification to determine its effect on proteasome activity is difficult. Nevertheless, the authors show that a specific treatment (venom phosphodiesterase-1 with S1 nuclease) changes the modification on some proteasome subunits *in vitro*. This change correlated with reduced proteasome activity. Summarizing these results, it suggests that treating cells with proteasome inhibitor has two effects. First, there is a direct inhibition resulting from inactivation of proteasome active sites. Second, there is an indirect inhibition, where bortezomib induces post-translational modifications on the proteasome that reduce proteasome activity.

This intriguing new model raises a whole set of new questions. What is the identity of the post-translational modification and what is the enzyme responsible? Are these more abundant in MM cells as compared with cells that are more resistant to bortezomib treatment? Identifying them would allow researchers to rigorously test the model by showing that cells are less sensitive to bortezomib upon elimination of the modification. Furthermore, it will allow researchers to test if this inhibitory proteasome modification is induced or increased specifically in cells sensitive for proteasome inhibitors. From a biochemical perspective, it is interesting that the presence of a proteasome inhibitor buried within the large enzyme can result in activation of an enzyme responsible for post-translational modifications. It has previously been shown that proteasome inhibitors induce structural changes in the proteasome

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(Arciniega et al., 2014; Kleijnen et al., 2007). These changes could trigger the recognition by enzymes responsible for the post-translational modification; however, this link between structural changes and induction of post-translational modifications remains to be shown.

One problem with bortezomib treatment in the clinic is the occurrence of drug resistance, for example through mutation in the bortezomib binding subunit PSMD5 (Dou and Zonder, 2014). However, not all causes of resistance have been determined. If patients acquire mutations that interfere with the ability to induce indirect inhibition as described here (Pitcher et al., 2015–in this issue), it would be predicted to cause resistance to bortezomib. Besides bortezomib, there are new proteasome inhibitor drugs under development, like the FDA-approved second generation proteasome inhibitor carfilzomib, for treating bortezomib-resistant patients and patients experiencing severe bortezomib-related side effects and toxicity (Dou and Zonder, 2014; Kubiczкова et al., 2014). It will be important to determine the extent to which the phenomena described by Pitcher et al. are specific for bortezomib, chymo-trypsin-like inhibitors, or all proteasome inhibitors.

In sum, the data by Pitcher et al. suggest that the presence of a strong indirect inhibition of proteasomes, as seen for MM cells, contributes to its ability to specifically kill MM cells and not healthy cells. If the post-translational modifications on the proteasome as described in this paper are responsible for the severe inhibition, the identification of the enzymes that modulate this modification will be interesting new drug targets to enhance effectiveness of proteasome inhibitors for various cancers.

Disclosures

The author declares no conflicts of interest

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